

VARIABILITY AMONG PSEUDOCERCOSPORA GRISEOLA ISOLATES BY ANASTOMOSIS AMONG HYPHAE

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INTRODUCTION: The fungus *Pseudocercospora griseola* is the causal agent of angular leaf spot, a disease of common bean (*Phaseolus vulgaris* L.). Yield losses can be as high as 80%. Breeding for disease resistance is the most effective strategy to control the bean angular leaf spot disease. The successful development of angular leaf spot-resistant cultivars depends on understanding the levels of variability among populations of the pathogen. Traditionally, the high pathogenic and genetic variation has been evaluated by differential cultivars and molecular markers. There is a need to explain the wide pathogenic and genetic variability. The parasexuality has been presented as a mechanism used by asexual fungi and for its occurrence the formation of anastomosis among hyphae is needed. Anastomosis, i.e. fusion between vicinal hyphae, represents an important communication within a fungal system that leads to the flow of cytoplasmic and genetic material and, consequently, to the increase genetic variability. The anastomosis occurrence among hyphae can be taken into consideration as a trait for population studies. The aim of this work was to identify the variation among isolates of *P. griseola* collected in Minas Gerais state, Brazil, by anastomosis groups.

MATERIALS AND METHODS: A total of 20 isolates of *P. griseola* collected in Minas Gerais State (Ijací, Lambarí and Lavras) and deriving from the culture collection of the Biology Department, Universidade Federal de Lavras (Lavras, MG, Brazil). Each isolate was confronted with itself and all other isolates according to a modified version of the method of Rodríguez-Guerra et al. (2003). Briefly, a sterilized microscope slide was placed on a Petri dish containing water-agar (2%) and covered with a thin layer of M3 medium. Fragments of isolates (5.0 mm) under confrontation were placed on the slide at a distance of 5.0 mm from each other and incubated for 15 days at 22°C. All confrontations were carried out at least in duplicate. Following incubation, the slide was lifted from the Petri dish and the original fungal fragments were carefully removed in order to leave only the newly formed hyphal masses. Hyphae were stained with a 0.05% solution of trypan blue-lactophenol, the slide was covered with a cover slip and submitted to examination under the light microscope. Anastomosis was classified as positive (compatible reaction) following observation of fusion of hyphae from the paired isolates.

RESULTS AND DISCUSSION: Anastomosis bridges were observed in H form (Figure 1). Anastomoses were observed for all the isolates (Table 1), however three isolates presented 85.0% of compatibility. The Pg-48 isolate presented smaller percentile (45.0%) of anastomosis formation with others isolates. The similarity estimates for the anastomosis data varied from 0.15 to 0.85, evidencing high variability with sixteen anastomosis groups being observed. All the groups were formed for a single isolate, except, one group that formed by Pg 01, Pg 02, Pg 07, Pg 08 and Pg 12 isolates. Isolates from different groups can present anastomosis among hyphae. This is the first report of the anastomosis occurrence among hyphae for the *P. griseola* fungus.

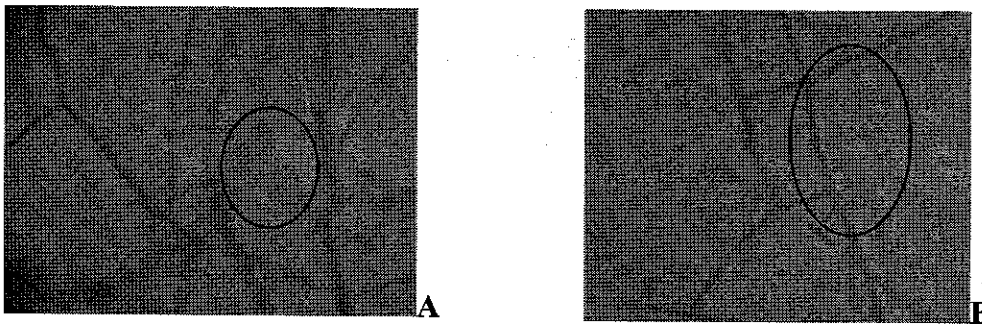


FIGURE 1. Anastomosis, in the form of an H-shaped fusion (circled), between hyphae of isolates Pg-01 and Pg-08 (A) and Pg-16 and Pg-52 (B).

TABLE 1. Compatibility reactions (anastomosis among hyphae) among isolates and proportion of compatible reaction (%) for each isolate.

Isolados	Pg 01	Pg 02	Pg 03	Pg 07	Pg 08	Pg 12	Pg 16	Pg 19	Pg 24	Pg 35	Pg 41	Pg 45	Pg 46	Pg 48	Pg 52	Pg 53	Pg 54	Pg 55	Pg 63	Pg 65	%
Pg-01	+	+	-	+	+	+	+	-	+	+	+	+	-	+	-	+	-	+	+	+	75,0
Pg-02		+	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	85,0
Pg-03			+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	75,0
Pg-07				+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	+	85,0
Pg-08					+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	85,0
Pg-12						+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	80,0
Pg-16							+	+	-	+	-	-	+	-	+	-	-	+	+	-	60,0
Pg-19								+	+	+	+	+	+	-	-	-	+	+	-	+	60,0
Pg-24									+	-	+	+	+	-	+	-	+	+	+	-	75,0
Pg-35										+	+	-	+	+	+	-	+	+	+	+	80,0
Pg-41											+	-	+	-	+	+	+	+	+	-	80,0
Pg-45												+	+	-	+	-	+	-	+	+	70,0
Pg-46													+	+	+	+	-	+	-	+	75,0
Pg-48														+	-	-	-	+	-	-	45,0
Pg-52															+	+	+	-	+	+	65,0
Pg-53																+	-	-	+	-	55,0
Pg-54																	+	-	+	-	65,0
Pg-55																		+	+	+	75,0
Pg-63																			+	+	80,0
Pg-65																				+	70,0

+ compatible reaction; - incompatible reaction.

CONCLUSIONS: High variability for anastomosis occurrence among hyphae and auser clustering among isolates for formation of anastomosis among hyphae demonstrating the existence of genetic variability for loci involved with the control of this trait.

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REFERENCE:

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